A Phylogenetic Analysis Identifies Heterogeneity Among Hepatocellular Carcinomas

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Primary hepatocellular carcinoma (HCC) is a significant cause of cancer morbidity and mortality on the global scale. Although epidemiologic studies have identified major risk factors for HCC, the sequence of oncogenic events at the molecular level remains poorly understood. While genetic allele loss appears to be a common event, the significance of the loss is not clear. In order to determine whether allele loss appears to be a random event among HCCs or whether patterns of loss cluster in groups of tumors, a phylogenetic approach was used to examine 32 tumors for genome-wide loss of heterozygosity employing 391 markers. Clusters identified by the phylogenetic analysis were then contrasted to compare candidate locus variation among individuals and to determine whether certain clusters exhibited higher loss rates than other clusters. The analysis found that 3 major and 1 minor cluster of loss could be identified and, further, these clusters were distinguished by variable rates of loss (cluster 1, 29%; cluster 2, 21%; cluster 3, 16%). The analyses also indicated that the allele loss rates in HCC were not insignificant and that the patterns of allele loss were complex. In addition, the results indicated that an individual's constitutional genotype at the EPHX1 locus may be a critical factor in determining the path of tumor evolution. In conclusion, it appears that in HCC, allele loss is not random, but clusters into definable groups that are characterized by distinctive rates of loss. (HEPATOLOGY 2002;36:1341-1348.)

Primary hepatocellular carcinoma (HCC) is the fifth most frequently occurring tumor in the world and the fourth most common cause of cancer mortality.[1,2] Epidemiologically, it is strongly associated with infection with hepatitis B (HBV) and hepatitis C viruses, aflatoxin B_1 (AFB_1) contamination of foodstuffs, alcohol consumption, and several genetic syndromes including hemochromatosis and Wilson's disease.[3] The sequence of events leading from risk factor exposure to the development of a hepatocellular tumor, however, is poorly understood. Although HBV has been shown to integrate in some tumors, integration has not been shown to be acutely transforming.[4] The mechanism of hepatocarcinogenesis of AFB_1, however, may be somewhat clearer in that a hotspot mutation in codon 249 of the p53 tumor suppressor gene has been linked to AFB_1 exposure in high-risk areas of the world.[5,6] In addition, evidence of genetic susceptibility to the effects of AFB_1 has been reported.[7]

At the molecular level, it appears that most types of cancer are characterized by genetic alterations involving oncogenes and/or tumor suppressor genes.[8] In HCC, the accumulated evidence suggests that widespread allele loss is a fairly common event. Loss of heterozygosity (LOH) studies have reported genetic loss on a wide variety of chromosomes, notably including the short arms of chromosomes 1, 9, and 17 and the long arms of chromosomes 4, 6, 13, and 16.[9-15] However, because LOH is frequently reported on a variety of chromosomal arms and no single tumor suppressor gene has been definitively implicated, interpretation of the accumulated LOH data is complex. This common allele loss in HCC is consistent with several explanations. It is possible that widespread genome disruption is necessary for hepatocarcinogenesis to occur.

Abbreviations: HCC, hepatocellular carcinoma; HBV, hepatitis B virus; AFB_1, aflatoxin B_1; LOH, loss of heterozygosity; HBsAg, hepatitis B surface antigen; STRP, simple tandem repeat polymorphism; GSTM1, glutathione-S-transferase M1; GST12, microsomal glutathione-S-transferase; GSTT1, glutathione-S-transferase theta; GSTP1, glutathione-S-transferase Pi; NQO1, NAD(P)H:quinone oxidoreductase; EPHX1, epoxide hydrolase 1; EPHX2, epoxide hydrolase 2.

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Alternatively, the involvement of more than one tumor suppressor gene may be necessary. Equally conceivable is the possibility that there may be several molecular pathways in hepatocarcinogenesis and what appears to be a complex pattern of loss, in aggregate, is simply the mixture of several different patterns. Therefore, we sought to determine whether patterns of LOH tended to cluster in groups of tumors by a phylogenetic analysis that used a high-resolution, high-heterozygosity panel of 391 markers. In addition, we questioned whether particular patterns of loss were more likely to be associated with high loss rates. Finally, we sought to examine whether patterns of loss were related to candidate locus variation among individuals. To examine this question, constitutional genetic variability in candidate loci was correlated with allele loss in the tumors.

Patients and Methods

Population. The study samples included paired tumor and normal tissue from 32 individuals with a diagnosis of primary HCC. All HCC patients were ethnic Chinese and were native to Haimen City, China. Haimen City is located on the north shore of the Yangtze River in China's Jiangsu Province. The area is a high-risk area for HCC and is know to have high rates of chronic infection with HBV (12% to 15% of the population) and to have, historically, had high levels of AFB_1 contamination of the food supply. Of the 32 HCC patients included in the study, 5 were women (15.6%). The mean age at diagnosis was 53.8 years and ranged between 33 and 74 years. Twenty-six individuals (81%) were seropositive for hepatitis B virus surface antigen (HBsAg) at diagnosis; 2 individuals (6%) were seronegative and 4 individuals were of indeterminate status. Eight of the patients (25%) reported having at least one first degree relative with a diagnosis of HCC.

Laboratory Analysis. The DNA was extracted from all 32 paired tumor and normal tissue samples using standard methods.[16] Genome-wide LOH was determined by typing the Weber/CHLC (version 6.0) marker panel, which is available online at http://lpqws.nci.nih.gov/html-chlc/ChlcMarkerMaps.html#WeberV6. The Weber/CHLC panel is composed of 391 genome-wide simple tandem repeat polymorphism (STRP) markers with an average resolution of 10 cM and an average heterozygosity of 0.76. Eighty-six percent of the markers are tri- or tetranucleotide repeat STRPs. STRP analysis was performed using the ABI 377XL semi-automated fluorescence electrophoresis instrument (Applied Biosystems, Foster City, CA), configured to run 66 lanes. Each marker was amplified by polymerase chain reaction with fluorescently tagged forward primers. The products were pooled (6 to 15 markers) and run on a 4% denaturing acrylamide gel with an internal size standard in each lane. After human inspection of the run, the resulting data were analyzed using GENOTYPER software (Applied Biosystems) that determined allele size, identification, and peak height. LOH was defined as a peak ratio of ≤ 0.5.

To determine the presence of the p53 codon 249 G→T transversion, analysis was performed using the previously published restriction endonuclease assay.[17] Candidate locus genotyping was performed as previously described.[7] The loci of interest were glutathione-*S*-transferase M1 (GSTM1), microsomal glutathione-*S*-transferase (GST12), glutathione-*S*-transferase theta (GSTT1), glutathione-*S*-transferase Pi (GSTP1), NAD(P)H:quinone oxidoreductase (NQO1), epoxide hydrolase 1 (EPHX1), and epoxide hydrolase 2 (EPHX2), and codon 249 of the p53 gene. The candidate loci were selected either because of a possible relationship to AFB_1 detoxification[7] or because, in the case of p53, a specific mutation had been shown to be related to HCC in high aflatoxin areas.[5,6]

Phylogenetic Analysis. To determine whether tumors evolve along distinct pathways over time, an evolutionary tree building algorithm was used to examine the data (Phylogeny Inference Package [PHYLIP] v3.57, provided courtesy of J. Felsenstein, University of Washington). The PHYLIP package assumes that evolution of events occurs over time and identifies the key early events that mark divergence of pathways. Markers informative in more than 10 tumors were analyzed by the Camin-Sokal algorithm as implemented by the MIX program. The robustness of the results was analyzed and a consensus tree was obtained by use of the CONSENSE program (CONSENSE, v. 3.5c, provided courtesy of J. Felenstein, University of Washington).

The PHYLIP analysis was based on the assumption that the progression of genetic changes in hepatocarcinogenesis is analogous to the evolution of various populations over evolutionary time. At the outset of a phylogenetic analysis of evolution of populations, there is a common ancestor. In the case of HCC, the phylogenetic analysis, utilizing the Camin-Sokal parsimony method, assumes that the common ancestor is no LOH present. Over time, as species diverge from the common ancestor, so would HCCs diverge into alternative pathways of allele loss. The key events in the divergent evolution would be the early allele loss events that characterize each branch of the evolutionary tree. The MIX program of the PHYLIP analysis package attempts to identify the divergence of the tumors using the fewest possible number of transitions (in this instance, LOH events) to alternative states. The pro-

gram was set to randomize the order of input of the tumors in 100 iterations to determine whether order of entry affected the outcome. The resulting set of plausible trees were then fed into the CONSENSE program to generate a final consensus tree based on the most commonly observed branches in the input trees. Finally, the consensus tree was fed back into the MIX program to reestimate the branch distances.

Statistical Analysis. To examine whether particular genotypes of candidate risk loci were associated with allele loss rates, analysis of variance and Student's *t*-test were used. Analysis of variance was also used to determine whether the genome-wide allele loss rates varied by the tree branches that resulted from the PHYLIP analysis. Finally, χ^2 analysis was used to examine whether different candidate locus risk allele distributions were associated with different tree branches. All data analyses were performed using the STATISTICA statistical analysis package (StatSoft, Tulsa, OK).

Results

The Weber/CHLC (version 6) panel was typed on all 32 tumor/normal pairs of tissue. Thirty of the markers did not amplify consistently and were not used, leaving a total of 361 typed markers. Among the remaining markers, the observed heterozygosity was 65% in this cross-ethnic sample set. The markers were spread across all autosomal chromosomes and the X chromosome. The number of markers per chromosome varied by size of the chromosome and ranged between 30 on chromosome 1 to 5 on chromosome 21. All chromosome arms showed loss at one or more loci. The allele loss rate of a marker was defined as the number of tumors showing loss of an allele when compared with the normal tissue counterpart, divided by the number of tumors informative at a particular locus. The allele loss rate of a tumor was defined as the number of markers showing loss of an allele when compared with the normal tissue counterpart, divided by the number of markers informative in that tumor.

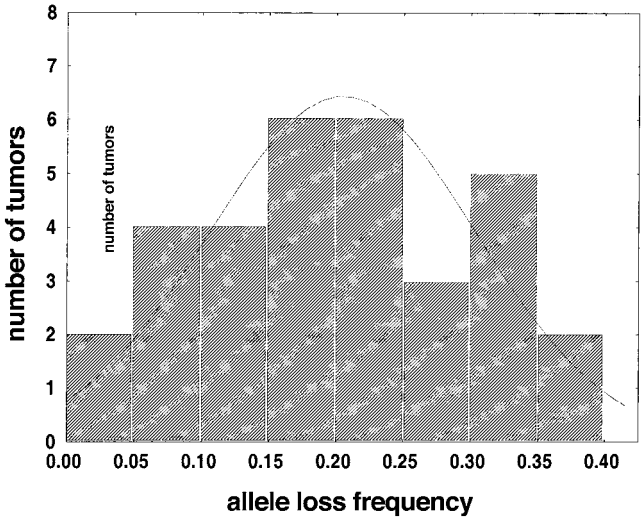

Fig. 1. HCC genome-wide allele loss rate by individual tumor. The **dotted line** represents the expected normal.

Table 1. Hepatocellular Carcinoma Allele Loss Profiles of Markers With Greater Than 50% Loss of Heterozygosity

Ratio	Marker	Chromosome	cM
0.75	028XV12	5	0
0.71	GATA64B04	17	29.7
0.67	GATA23D06	8	20.3
0.65	GATA85F06	6	67.9
0.64	GATA11E09	4	140.9
0.62	GGAA8D08	6	182.4
0.62	GATA11C06	16	122.2
0.62	GGAA19H07	4	175.9
0.60	309VA9	13	119.5
0.59	ACTC1	15	26.5
0.58	GATA81D12	16	83.5
0.56	GATA10H07	17	33.2
0.56	GATA2A12	23	

Similar to other reports, the allele loss rate per tumor was extremely variable. At the lowest end, 2 tumors showed less than 5% loss, whereas at the opposite extreme, 2 tumors showed greater than 35% loss. Across all tumors, the average allele loss rate was 20% (Fig. 1). The loci showing greater than 50% loss are shown in Table 1. The large number of loci reported in Table 1 and the apparently not normal distribution of loss events suggest the existence of more than a single etiology of HCC. Among all markers, the locus showing the greatest allele loss rate at 75% was AFM028XB12 on chromosome 5p. The only other marker showing greater than 70% loss was GATA64B04 (71%) on chromosome 17p near the p53 region. Other makers showing a high rate of loss were GAT23DO6 (67%) on chromosome 8p, GATA85F06 (65%) and GGAA8D08 (62%) on chromosome 6p and 6q, respectively, GATA11E09 (64%) and GGAA19H07 (62%) on chromosome 4q, 309V0A9 (60%) on chromosome 13q, ACTC1 (59%) on chromosome 15p, GATA81D12 (58%) on chromosome 16q, GATA10H07 (56%) on chromosome 17p, and GATA2A12 (56%) on chromosome Xp. The complete LOH results for all markers, by chromosome, can be accessed on the Internet at http://www.lpg.nci.nih.gov/hcc_loh.

Because of the great variability in allele loss rate, we questioned whether individuals with particular alleles at candidate loci experienced higher rates than individuals with other allelotypes. We also questioned whether individuals with a p53 codon 249 mutation had allele loss

rates that varied from other individuals. A series of *t*-tests revealed that there was no significant association between alternate alleles of NQO1, EPHX2, EPHX1, GSTM1, and GSTT1 and higher allele loss rates. In contrast, individuals with at least one GSTP1 variant allele a had significantly higher allele loss rate ($P = .01$) as did individuals with one or more GST12 variant alleles ($P = .03$). Given the number of contrasts, however, the relationship of GSTP1 and GST12 allelotypes to allele loss rate is questionable. The relationship of a p53 codon 249 mutation to higher allele loss rate, however, remained statistically significant after adjustment for multiple comparisons ($P = .002$).

To determine whether the complex loss patterns were the result of etiologic heterogeneity or simply represented generalized random disruption of the genome, we performed phylogenetic analysis of the 32 tumors. This analysis was based on the assumption that the progression of genetic changes in hepatocarcinogenesis is analogous to the evolution of various populations over evolutionary time. At the outset of a phylogenetic analysis of evolution of populations, there is a common ancestor. In the case of HCC, the phylogenetic analysis, utilizing the Camin-Sokal parsimony method, assumes that the common ancestor is no LOH present. Over time, as species diverge from the common ancestor, so would HCCs diverge into alternative pathways of allele loss. The key events in the divergent evolution would be the early allele loss events that characterize each branch of the evolutionary tree. The MIX program of the PHYLIP analysis package attempts to identify the divergence of the tumors by using the fewest possible number of transitions to alternative pathways. The program was set to randomize the order of input of the tumors in 100 iterations to determine whether order of entry affected the outcome. The resulting set of plausible trees were then fed into the CONSENSE program to generate a final consensus tree based on the most commonly observed branches in the input trees. Finally, the consensus tree was fed back into the MIX program to reestimate the branch distances.

The phylogenetic analysis (Fig. 2) found that the tumors could be grouped into 3 major evolutionary branches and a fourth minor branch. A measure of the robustness of the result was the fact that PHYLIP reproducibly regenerated the same branches regardless of the order of tumor entry. The tumors (n = 9) in the first branch were characterized by LOH at 5 markers; 3 markers on proximal 4q, 1 marker on 13q, and 1 marker on 17p. The 4q markers were GATA2F11, GATA11E09, and GATA8A05. The 13q marker was GATA6B07 and the 17p marker was GATA10H07.

The second branch of the phylogenetic tree (n = 10) shared a single common allele loss site; ATA26B08 on chromosome 4q. The tumors of the third branch (n = 10) shared 2 sites of LOH: marker AFM028XB12 on chromosome 5p and GGAA20C10 on chromosome 8p. The final branch of the tree included 3 tumors that shared no common LOH sites with the three other branches and no common sites with each other.

To determine whether the 3 major branches varied by demographic descriptors or risk factors, the individuals in each branch were contrasted. There was no difference by branch in sex distribution, mean age at diagnosis (branch 1, 50 years; branch 2, 53.5 years; branch 3, 54.7 years) or positive family history of HCC (branch 1, 25%; branch 2, 25%; branch 3, 33%). In addition, there was no evidence to suggest that the 3 branches varied by exposure to risk factors. The HBsAg seropositivity rate did not significantly differ by branch (branch 1, 89%; branch 2, 100%; branch 3, 86%). In addition, the percentage of individuals having the G→T transversion in codon 249 of p53 also did not vary by branch, perhaps suggesting that exposure to aflatoxin did not differ significantly among individuals.

Once the tumors were grouped into branches, we sought to determine whether there was a difference, by branch, in the allele loss rates. Analysis of variance found that a statistically significant difference was apparent ($F_{df(2,25)} = 5.27$, $P = .01$). The tumors in branch 1 had an average allele loss rate of 29%, the branch 2 tumors had an average allele loss rate of 21%, and the branch 3 tumors had an average allele loss rate of 16%.

In addition to examining allele loss rate, genotypes of candidate loci were contrasted among the 3 major tree branches. A comparison of allelotypes of GSTM1, GST12, GSTT1, GSTP1, NQO1, EPHX1, and EPHX2 among the branches found that individuals in branch 1

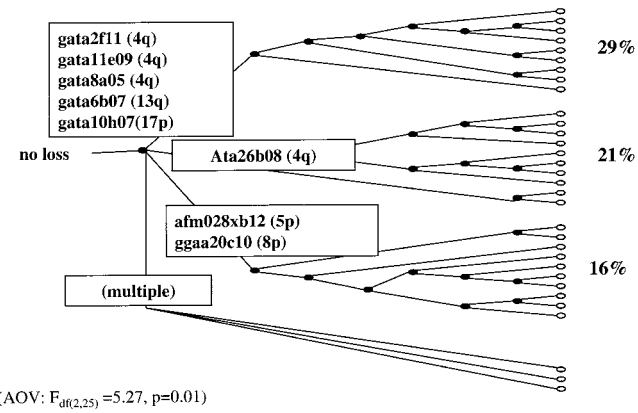

Fig. 2. HCC tumor lineages. Allele loss clusters and allele loss rates.

were significantly more likely to have at least one high risk EPHX1 allele than were the individuals in either the second or third arm ($t = -2.14$, $P = .04$). Thirty-three percent of the individuals in branch 1 had at least one high-risk EPHX1 allele, as opposed to 0% in branch 2 and 11% in branch 3. In contrast, there was no statistically significant difference in allelotypes of the other 7 loci across the branches.

Discussion

Numerous studies have examined LOH in HCC, with the great majority focusing on specific loci or chromosomal arms. In this article, we describe a high-density, high-heterozygosity, genome-wide investigation of LOH that incorporates the largest number of markers reported to date. The genome-wide aspect is particularly critical in HCC in that, as we show here, hepatocellular tumors tend to be characterized by generalized genomic disruption. This disruption results in multiple regions of LOH, separated by regions of no LOH, so even low-resolution genome-wide studies may not detect the same patterns that are detectable with a high-resolution approach.

Our results show that LOH in HCC is not random in that consistent patterns of loss can be identified by means of a phylogenetic analysis. As anticipated, the allele loss rate in HCC was not insignificant, and the patterns of loss tended to be complex. The clustering of loss events indicates that there may be heterogeneity in hepatocarcinogenesis. For example, the development of some tumors seems to require loss in certain areas of chromosome 4q and accompanying loss on chromosomes 17p and 13q (branch 1 tumors). Other tumors, however, seem to develop after loss on chromosomes 5p and 8p (branch 3 tumors). Still another group of tumors (branch 2) is characterized by loss on 4q in a region that is distinct from the regions that are important in the branch 1 tumors. Among all 3 branches, the branch 3 tumors seem to require the fewest overall loss events, in that they have a mean loss rate of 16% versus 29% in the branch 1 tumors and 21% in the branch 2 tumors.

Whether different exposures and/or susceptibilities can account for any of the differences in the pathways is unclear. It does not appear that hepatitis B viral infection, however, distinguishes the branches, because there was no significant difference in HBsAg seropositivity. The branches were also not distinguishable by mean age at diagnosis, sex, or family history of HCC. All tumors came from an AFB_1 endemic area of the Jiangsu Province in China; however, AFB_1 exposure could not be directly assessed on an individual basis. However, the fact that the individuals in branch 1 were significantly more likely to have at least one EPHX1 high-risk allele may indicate that AFB_1-related hepatic damage is more common in branch 1 hepatocarcinogenesis. As has been previously shown,[7] the presence of one or more high-risk EPHX1 alleles correlates with AFB_1-albumin adducts among individuals living in AFB_1-endemic areas.

In previous studies that did not use a genome-wide approach, the chromosomal arms most commonly found to have suffered loss include 1p, 4q, 6q, 8p, 9p, 13q, 16q, and 17p.[10,18-23] The tumor suppressor loci known to be present on these arms include Nb (1p36.3), M6P/IGF2r (6q26), CDKN2A (9p21), Rb1 (13q), and p53 (17p13.1). The significance of these losses, however, has been difficult to assess in that LOH appears to be such a common event in hepatocarcinogenesis. A genome-wide approach offers the inarguable advantage of being able to identify LOH without having *a priori* hypotheses concerning particular loci. The reported genome-wide studies of LOH are fewer in number than the non–genome-wide studies but have, for the most part, tended to implicate the same chromosomal arms as the non–ge-

Table 2. Prior Genome-Wide Loss of Heterozygosity Studies of Hepatocellular Carcinoma

Ethnicity	Reference	No. of HCC	No. of Markers	Percent Loss at Chromosomal Location																
				1p	1q	4q	5p	5q	6p	6q	8p	8q	9p	9q	11p	13q	15p	16p	16q	17p	Xp
French*	24	48	275	44		42				35	60		30			29		40	39	48	
Several	13	12	24			80															
Japanese	30	46	44					45							45				35	55	
U.S.	25	28	28	63		46										29				29	
Japanese	28	15	18		29						35	35		45				33	33		
Chinese	7	32	361			64	75		65	62	67					60	59		58	70	56
Japanese*	26	120	195	26	20	40				36	42	23	21	20		30		22	28	33	
Japanese	27	44	216			48				36	56					31			43	54	
Korean	14	22	68		68	73					64	77				40				46	
Japanese	31	68	16															22	39		
Taiwanese	29	34	232										21			32			41	32	
Chinese	58	104	382	76	66	75				56	67		72			73			65	73	

*Ethnicity presumed.

Table 3. Comparative Genomic Hybridization Studies of Hepatocellular Carcinoma

First Author (Reference)	Origin of Tumors	No. of HCC	Chromosomal Arm Location											
			1p	1q	4q	6p	6q	8p	8q	13q	16q	17p	17q	20q
Marchio (34)	Several	50	Loss	Gain	Loss	Gain	Loss	Loss	Gain	Loss	Loss	Loss	Gain	
Kusano (32)	Japan	41		Gain	Loss			Loss	Gain	Loss	Loss	Loss		
Wong (35)	Hong Kong	67		Gain	Loss			Loss	Gain	Loss	Loss		Gain	Gain
Guan (36)	Hong Kong	50	Loss	Gain	Loss			Loss	Gain		Loss	Loss		Gain
Tornillo (37)	Italy, Switzerland	41	Loss	Gain	Loss	Gain	Loss	Loss	Gain	Loss			Gain	
Marchio (33)	Not stated	34	Loss	Gain		Gain	Loss	Loss	Gain	Loss			Gain	
Wong (38)	Several	83		Gain	Loss			Loss	Gain	Loss	Loss	Loss		
Chen (39)	Taiwan	31		Gain	Loss	Gain		Loss	Gain	Loss	Loss		Gain	Gain
Balsara (40)	China, U.S.	52		Gain	Loss			Loss	Gain		Loss	Loss		
Shiraishi (41)	Japan	31		Gain				Loss	Gain	Loss	Loss	Loss		
Niketeghad (42)	Germany	21		Gain	Loss		Loss		Gain	Loss	Loss		Gain	Gain
Koo (43)	South Korea	24		Gain	Loss				Gain	Loss	Loss	Loss		

nome-wide studies (Table 2). The arms most frequently implicated by the genome-wide studies include 4q, 8p, 13q, 16q, and 17p.[7,13,14,24-31] In addition to the high-density allelotype studies, at least 12 comparative genomic hybridization studies have now been reported and, again, have found chromosomal loss in many of the same areas as the LOH studies (Table 3).[32-36] Ten of the 12 studies reported loss on chromosomes 4q,[32,34-43] 8p,[32-41] and 16q.[32,34-36,38-43] Demonstrating striking consistency, all 12 studies reported gain on chromosomes 1q and 8p.[32-43]

In the data reported here, the markers most commonly lost are consistent with earlier reports in the literature. Loss of regions of chromosome 4q has been reported in tumors from the United States,[13,25] Korea,[44] Taiwan,[45] China,[18] Japan,[15,26,27,46] France,[24] and Italy.[47] Tumors showing LOH on 4q have been reported to be more advanced,[46] to be more likely to have a p53 codon 249 mutations,[18] to be associated with increased α-fetoprotein levels,[45] and to show loss in at least 7 different regions.[44]

Tumors having 13q loss have been reported to be associated with HBV infection[48,49] and to be more common at later stages of tumor development.[46] Previous reports of 13q loss in tumors from the city neighboring Haimen City, Qidong City, have reported high frequency loss in the regions including both the Rb1 tumor suppressor gene at 13q14[28,50] and the BRCA2 gene at 13q12.3.[18] Reports of HCCs from Taiwan have found similar results.[49] The 13q marker that helped define the branch 1 tumors in this report, GATA6B07, is in the region of Rb1. By contrast, the 13q marker with the highest frequency of allele loss, 309VA9, is located far distal to the Rb1 locus on 13q.

LOH in the p53 region (chromosome 17p13.1) has been widely reported in HCC.[15,18,20,22,50-52] Among the tumors reported here, the 17q marker with the greatest loss rate, GATA64B04, is in the p53 region. The marker that helped defined the branch 1 tumors, however, was slightly proximal to that. In other reports, 17p loss was associated with HBV infection,[10] larger size,[52] and more advanced tumor stage.[20,22]

Based on the accumulated evidence in the literature, it is conceivable that the branch 1 tumors are more advanced in stage, associated with HBV infection, and associated with p53 codon 249 mutations. The evidence that the individuals in this branch are more likely to have one or more high-risk EPHX1 alleles may also indicate that these tumors arise in persons genetically susceptible to the effects of AFB_1. By contrast, no individual in branch 2 had a high-risk EPHX1 allele. The branch 2 tumors were characterized by loss of a different region of 4q than the regions lost in the branch 1 tumors and showed an overall lower rate of allele loss than did the branch 1 tumors.

The chromosomal regions characteristic of branch 3 have also been investigated in previous studies. Loss on 8p has been widely reported in HCC, particularly in the regions of 8p21-22 and 8p23.[20,22,26,53-55] Fujiwara et al.[56] reported the isolation of a candidate tumor suppressor gene in the region 8p21.3-22 (PDGF-receptor beta–like tumor suppressor), but further evidence suggests that the locus may not be related to the development of HCC.[57]

Taken as a whole, these analyses suggest that HCC is characterized by molecular heterogeneity. HCC tumors may evolve down a variety of pathways and the pathways are subject to a greater or lesser allele loss rate. The results also indicate that an individual's constitutional genotype at certain candidate loci may be critical in determining the path of tumor evolution. The genome-wide examination of a greater number of tumors and the subsequent phylogenetic analysis should enable us to determine, with more precision, the exact loss events that are critical to each

evolutionary step. The determination of these steps may then open new leads to examine in identifying the causal events in hepatocarcinogenesis.

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